Independent claim 38 has been amended to more clearly define the invention as genetically engineering a cell to produce both a PHA synthase and a fatty acid:acyl Co A transferase. Support can be found at page 4, lines 4-11, and pages 9-10.

Claims 53-56 have been amended to clarify that the cells are genetically engineered to express additional enzymes, rather than merely producing the enzymes.

Claim 64 has been added. Claim 64 defines the method step of claim 38 as originally filed wherein the polyester is separated from the genetically engineered cells.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 38-61 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

A description as filed is presumed to be adequate, unless and until the examiner establishes a *prime facie* case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed. (See, e.g., *In re Marzocchi*, 439 F. 2d 220, 224, 169 USPQ 367, 370 (CCPA 1971), MPEP §2163.04).

In stating that the specification only teaches one PHA synthase gene and one fatty acid acyl-CoA transferase gene, the examiner appears to have overlooked much of the specification. One does not have to have actually reduced to practice all representatives of a species or genus to be entitled to claim the species or genus. The specification teaches a wide variety of PHA

synthase genes, from numerous organisms, all of which are known and publicly described in the literature and in Genebank (see, e.g., p. 9, lines 5-15 of the specification) and a wide variety of fatty acid acyl-CoA transferase gene, from numerous sources, all of which are known and publicly described in the literature and Genebank (see, e.g., p. 9, lines 15-25 of the specification). A search of MedLine yields many abstracts detailing the cloning and characteristics of PHA synthases from a wide variety of bacteria, including *Alcaligenes, Pseudomonas, Bacillus, Cyanobacteria, Thiocapsa, Chromatium, Rhodospirillum*, and others. There is an even broader source of fatty acid acryl-CoA transferase genes available based on MedLine search. See also Heibuchel, Review Adv. Biochem. Eng. Biotechnol. 71:81-123 (2001).

The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species. (See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406). A "representative number of species" means that the species which are adequately described are representative of the entire genus. There may be situations where even one species can adequately supports a genus (see, e.g., *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326-27; In re *Herschler*, 591 F.2d at 1214, 211 USPQ at 326-27). When there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. (See, e.g., MPEP §2163) The present specification teaches more than one species for both genus.

Claims 38-61 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. This appears to be the same rejection. For the reasons discussed above, Applicants believe that the examples in the present specification together with a statement applicable to the genus as a

whole is sufficient so that one skilled in the art (in view of level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation, and would need no further written description of the required genes. Proof of enablement should be required for other members of the genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation. The Examiner did not advance such reasons. (See, e.g., MPEP §2164.02)

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 38-61 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The objected to phrase "cell capable" or "the cell is further capable" has been deleted from the claims.

Rejection Under 35 U.S.C. § 102

Claims 38-42 and 44-61 were rejected under 35 U.S.C. § 102(a) as being anticipated by US Patent No. 6,117,658 to Dennis et al. ("Dennis"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Claimed Invention

As the claims now clearly define, polyesters are made in cells which express a PHA synthase, which have been genetically engineered to express a recombinant fatty acid:acyl CoA

transferase. Dependent claims further define other modifications of the cells to express other enzymes, or selection of the cell to express other enzymes.

Dennis

Dennis describe a process for producing a polyhydroxyalkanoate comprising 4-hydroxybutyrate monomer units, the method combining the PHA biosynthetic pathway with a succinic semialdehyde metabolic pathway that metabolizes succinic semialdehyde via a 4-hydroxybutyryl-CoA intermediate in order to produce high levels of PHA comprising 4HB monomer units. The "PHA biosynthetic pathway" comprises a PHA synthase.

The "succinic semialdehyde metabolic pathway" is so named because it produces succinic semialdehyde (Column 8, lines 44-45 of Dennis). The succinic semialdehyde metabolic pathway" preferably includes the full succinyl-CoA metabolic pathway, containing a 4-hydroxybutyrate dehydrogenase (4hbD), a succinic semialdehyde dehydrogenase (sucD), and an acetyl-CoA:4-hydroxybutyrate CoA transferase, or a pathway that produces a succinic semialdehyde (see column 8, lines 33-46, column 9, lines 21-28, and Fig. 1 of Dennis). The "succinic semialdehyde metabolic pathway" begins by reducing succinyl-CoA to succinic semialdehyde and CoA by succinic semialdehyde dehydrogenase (Column 8, lines 48-51, and Fig. 1 of Dennis). There is no disclosure of genetically engineering the organisms to express a fatty acid:acyl Co transferase. Acetyl-CoA:4-hydroxybutyrate CoA transferase is not part of a "succinic semialdehyde metabolic pathway", since the enzyme by itself cannot produce succinic semialdehyde. Accordingly, Dennis does not disclose the claimed process.

Rejection Under 35 U.S.C. § 103

Claims 38-39 and 41-43 were rejected under 35 U.S.C. § 103(a) as obvious over Dennis, in view of US Patent No. 5,512,468 to Greener ("Greener"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Greener

Greener describes the transformation efficiencies of *E. coli* strain XL1-Blue cells.

Dennis has been discussed above. There was no motivation or suggestion to combine Dennis and Greener. Dennis expressed the combination of the pathways and achieved the production of PHA in a non- XL1-Blue *E. coli* strain (see, e.g., column 23, lines 41-44). Dennis does not suggest a different *E. coli* strain should be used, even though Greener had already described XL1-Blue, and its transformation efficiencies. Greener never teaches the application of XL1-Blue in the production of PHA. In addition, there was no reasonable expectation of success for using the combination in the production of PHA. The production of recombinant enzymes is determined by many characteristics of the host cells. High transformation efficiency does not necessarily result in an increased production of enzymes.

In addition to the above reasons, claims 50-51 should be patentable over Dennis, because Dennis teaches away from producing PHA with 1,4-butanediol, 4-hydroxybutyric acid, and does not teach molecular oxygen in the culture medium. Dennis states that " [p]referably, the carbon source does not contain significant amounts of a structurally related precursor of 4-hydroxybutyrate, which means carbon sources that have the four carbon skeleton of 4-hydroxybutyrate and have a hydroxyl group on the fourth carbon. Examples of structurally

related precursors of 4-hydroxybutyrate include 4-hydroxybutyrate, 1,4-butanediol or 4-butyrolactone" (see column 10, lines 11-17 of Dennis). Dennis states that PHA "are synthesized by bacteria when carbon source levels are high and other nutritional necessities, such as nitrogen, phosphate, oxygen, or sulfur, are limited" (see, column 1, lines 22-28 of Dennis). This is the only place where oxygen is mentioned in Dennis.

Claims 52, 53, and 56 are also not anticipated by nor obvious over Dennis. With respect to claim 52, Dennis does not teach a recombinant protein capable of hydrolysing a lactone to the corresponding hydroxyalkanoic acid. With respect to claim 53, Dennis does not teach a recombinant 2-oxyglutarate decarboxylase protein. With respect to claim 56, Dennis does not teach any of a recombinant 2-methylcitrate synthase protein, a recombinant 2-methylcitrate dehydratase protein, a recombinant 2-methylisocitrate dehydratase protein, and a recombinant 2-methylisocitrate lyase protein.

Allowance of claims 38-61, and 64 is earnestly solicited.

Respectfully submitted,

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U.S.S.N. 09/380,773
Filed: September 03, 1999
AMENDMENT AND RESPONSE TO OFFICE ACTION

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that the enclosed Amendment and all documents shown as being attached is being facsimile transmitted to the U. S. Patent and Trademark Office on the date shown below.

Date: February 22, 2002

Aisha Wyatt

Marked Up Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

38. (Amended) A method for the preparation of a polyester, comprising the steps of:

[a) obtaining a cell capable of producing

i) a polyhydroxyalkanoic acid synthase protein; and

ii) a fatty acid:acyl-coenzyme A transferase protein;

b) establishing a culture of the cell;

c)] culturing [the cell] recombinant cells under conditions suitable for the production of the polyester[; and

isolating the polyester from the cell] , wherein the recombinant cells express a polyhydroxyalkanoic acid synthase protein and have been genetically engineered to express a fatty acid:acyl-coenzyme A transferase protein.

39. The method of claim 38, wherein the cell is a plant cell, mammalian cell, insect cell, fungal cell, or bacterial cell.

40. The method of claim 39, wherein the cell is a plant cell.

41. The method of claim 39, wherein the cell is a bacterial cell.

42. The method of claim 41, wherein the cell is *Escherichia coli*.

43. The method of claim 42, wherein the bacterial cell is *Eschrichia coli* strain XL1-Blue.

44. The method of claim 38, wherein the polyhydroxyalkanoic acid synthase protein is a polyhydroxyalkanoic acid synthase protein from *Alcaligenes eutrophus*.

45. The method of claim 44, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein is encoded by the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase structural gene.

46. The method of claim 38, wherein the fatty acid:acyl-coenzyme A transferase protein is a 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

47. The method of claim 46, wherein the 4-hydroxybutyrate:acyl-coenzyme A transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

48. The method of claim 47, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein is encoded by *Clostridium kluyveri orfZ* 4-hydroxybutyrate:acyl-coenzyme A transferase structural gene.

49. The method of claim 38, wherein the culture contains glucose.

50. (amended) The method of claim 38, wherein the culture contains <u>materials selected from the group consisting of</u> 4-hydroxybutyric acid, the sodium salt of 4-hydroxybutyric aicd, γ-butyrolactone, 1,4-butanediol, 4-hydroxyvaleric acid, γ-valerolactone, 1,4-pentanediol, 3-hydroxybutyric acid, the sodium salt of 3-hydroxybutyric acid, a hydroxypropionic acid, a hydroxybutyric acid, a hydroxyvaleric acid, a hydroxycaproic acid, a hyroxyheptanoic acid, a hydroxyoctanoic acid, a hydroxydecanoic acid, γ-caprolactone, γ-heptanolactone, γ-octanolactone, or γ-decanolactone.

51. The method of claim 38, wherein the culture contains molecular oxygen.

52. (amended) The method of claim 38, wherein the cell is [further capable of producing] genetically engineered to express a heterologous protein capable of hydrolysing a lactone to the corresponding hydroxyalkanoic acid.

53. (amended) The method of claim 38, wherein the cell is [further capable of producing] genetically engineered to express a heterologous 2-oxyglutarate decarboxylase protein and a heterologous 4-hydroxybutyrate dehydrogenase protein.

54. (amended) The method of claim 38, wherein the cell is [further capable of producing] genetically engineered to express a heterologous protein selected from the group consisting of a 2-methylcitrate synthase protein, a 2-methylcitrate dehydratase protein, 2-methylisocitrate dehydratase protein, 2-methylisocitrate lyase protein, a succinate:acetyl-Co A transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

55. (amended) The method of claim 38, wherein the cell is [further capable of producing] genetically engineered to express a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

56. (amended) The method of claim 38, wherein the cell is [further capable of producing] genetically engineered to express a 2-methylcitrate synthase protein, a 2-methylcitrate dehydratase protein,a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate lyase protein, a succinate:acetyl-Co A transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

57. The method of claim 38, wherein the polyester is a homopolyester.

58. The method of claim 57, wherein the homopolyester is poly(4-hydroxybutyric acid).

59. The method of claim 57, wherein the homopolyester is poly(3-hydroxybutyric acid).

60. The method of claim 38, wherein the polyester is a copolyester.

61. The method of claim 60, wherein the copolyester is poly(3-hydroxybutyric acid -co-4-hydroxybutyric acid).

Please cancel claims 62-63.

Please add the following new claim 64.

64. (New) The method of claim 38 further comprising separating the polyester from the recombinant cells.

U.S.S.N. 09/380,773
Filed: September 03, 1999
AMENDMENT AND RESPONSE TO OFFICE ACTION

Clean Version of Amended Claims
Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

38. (Amended) A method for the preparation of a polyester, comprising the steps of: culturing recombinant cells under conditions suitable for the production of the polyester, wherein the recombinant cells express a polyhydroxyalkanoic acid synthase protein and have been genetically engineered to express a fatty acid:acyl-coenzyme A transferase protein.

39. The method of claim 38, wherein the cell is a plant cell, mammalian cell, insect cell, fungal cell, or bacterial cell.

40. The method of claim 39, wherein the cell is a plant cell.

41. The method of claim 39, wherein the cell is a bacterial cell.

42. The method of claim 41, wherein the cell is *Escherichia coli.*

43. The method of claim 42, wherein the bacterial cell is *Eschrichia coli* strain XL1-Blue.

44. The method of claim 38, wherein the polyhydroxyalkanoic acid synthase protein is a polyhydroxyalkanoic acid synthase protein from *Alcaligenes eutrophus.*

45. The method of claim 44, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein is encoded by the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase structural gene.

46. The method of claim 38, wherein the fatty acid:acyl-coenzyme A transferase protein is a 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

47. The method of claim 46, wherein the 4-hydroxybutyrate:acyl-coenzyme A transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

48. The method of claim 47, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein is encoded by *Clostridium kluyveri orfZ* 4-hydroxybutyrate:acyl-coenzyme A transferase structural gene.

49. The method of claim 38, wherein the culture contains glucose.

50. (amended) The method of claim 38, wherein the culture contains materials selected from the group consisting of 4-hydroxybutyric acid, the sodium salt of 4-hydroxybutyric aicd, γ-butyrolactone, 1,4-butanediol, 4-hydroxyvaleric acid, γ-valerolactone, 1,4-pentanediol, 3-hydroxybutyric acid, the sodium salt of 3-hydroxybutyric acid, a hydroxypropionic acid, a hydroxybutyric acid, a hydroxyvaleric acid, a hydroxycaproic acid, a hyroxyheptanoic acid, a hydroxyoctanoic acid, a hydroxydecanoic acid, γ-caprolactone, γ-heptanolactone, γ-octanolactone, or γ-decanolactone.

51. The method of claim 38, wherein the culture contains molecular oxygen.

52. (amended) The method of claim 38, wherein the cell is genetically engineered to express a heterologous protein capable of hydrolysing a lactone to the corresponding hydroxyalkanoic acid.

53. (amended) The method of claim 38, wherein the cell is genetically engineered to express a heterologous 2-oxyglutarate decarboxylase protein and a heterologous 4-hydroxybutyrate dehydrogenase protein.

54. (amended) The method of claim 38, wherein the cell is genetically engineered to express a heterologous protein selected from the group consisting of a 2-methylcitrate synthase protein, a 2-methylcitrate dehydratase protein, 2-methylisocitrate dehydratase protein, 2-methylisocitrate lyase protein, a succinate:acetyl-Co A transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

55. (amended) The method of claim 38, wherein the cell is genetically engineered to express a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

56. (amended) The method of claim 38, wherein the cell is genetically engineered to express a 2-methylcitrate synthase protein, a 2-methylcitrate dehydratase protein,a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate lyase protein, a succinate:acetyl-Co A transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

57. The method of claim 38, wherein the polyester is a homopolyester.

58. The method of claim 57, wherein the homopolyester is poly(4-hydroxybutyric acid).

59. The method of claim 57, wherein the homopolyester is poly(3-hydroxybutyric acid).

60. The method of claim 38, wherein the polyester is a copolyester.

61. The method of claim 60, wherein the copolyester is poly(3-hydroxybutyric acid –co-4-hydroxybutyric acid).

64. (New) The method of claim 38 further comprising separating the polyester from the recombinant cells.